Epidermal HMG CoA Reductase Activity in Essential Fatty Acid Deficiency: Barrier Requirements Rather Than Eicosanoid Generation Regulate Cholesterol Synthesis

Ehrhardt Proksch, Kenneth R. Feingold, and Peter M. Elias

Department of Dermatology (EP), University of Kiel, Germany; Dermatology and Medical Services (KRF), Veterans Administration Medical Center; and Department of Dermatology (PME), School of Medicine, University of California, San Francisco, California, U.S.A.

We showed previously that the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis, increases after both barrier disruption with organic solvents and in essential fatty acid deficiency (EFAD). Here, we treated EFAD hairless mice with linoleic acid, columbinic acid (C18:3,n-6, trans; not metabolizable to known regulatory eicosanoids), prostaglandin E_2 (PGE_2), or latex occlusion, and determined transepidermal water loss (TEWL), epidermal protein content, and epidermal HMG CoA reductase activity. Increased TEWL rates in EFAD were accompanied by increased HMG CoA reductase activity (+130%, $n = 6$, $p < 0.01$) and protein content (+69%; $n = 6$, $p < 0.025$). Artificial restoration of the barrier by occlusion reduced the increase in enzyme activity and protein content toward normal, but barrier function, measured immediately after removal of the latex wrap, deteriorated further (TEWL: two-fold greater than EFAD unoccluded; $p < 0.01$). Topical applications of either linoleate or columbinate (but not PGE_2), normalized barrier function, HMG CoA reductase activity, and protein content. These results show that a) barrier function modulates HMG CoA reductase activity; b) reduction of cholesterol synthesis with occlusion results in a further deterioration in barrier function, suggesting that increased synthesis is a protective homeostatic response; and c) the barrier abnormality reflects a requirement for specific fatty acids for the barrier rather than resulting from epidermal hyperplasia or decreased prostaglandin generation. *J Invest Dermatol 99:216–220, 1992*

Prior studies have shown that essential fatty acid deficiency (EFAD) results in chronic barrier disruption, as measured by excess transepidermal water loss (reviewed in [1–3]). This chronic barrier abnormality is associated with epidermal hyperplasia and increased DNA synthesis [4,5]. The essential component missing from a EFAD-diet has been identified as linoleic acid [3,6,7] and either topical or systemic administration of linoleic acid restores barrier function and normalizes transepidermal water loss (TEWL). The defective barrier in EFAD-animals is attributable to substitution of oleate for linoleate in 0-acylsphingolipids [6]. Most epidermal linoleate is ester-linked to omega-hydroxy-acids, which in turn are amide-linked to sphingosine (ceramide I) [6,7]. It has been suggested that this molecule is critical for lamellar bilayer formation in the stratum corneum, and hence for maintenance of the permeability barrier [6,7]. It has also been demonstrated that linoleic acid normalizes the increased DNA synthesis and thereby reverses epidermal hyperplasia [4,5]. We recently have shown [5] that epidermal DNA synthesis is regulated by barrier function, suggesting that in EFDA the epidermal hyperplasia may be a physiologic attempt to repair the disturbed permeability barrier.

The mechanism by which EFAD and linoleate replenishment affect DNA synthesis and epidermal hyperplasia is not fully understood. Linoleate is a precursor of arachidonic acid, which in turn is a precursor for the production of prostaglandins, prostacyclines, and the thromboxanes by the fatty acid cyclooxygenase system [8]. It has been reported that topical applications of prostaglandin E_2 alleviate skin scaliness and hyperplasia in EFA deficient rat paws [9], but this treatment does not restore barrier function [3,10–12]. It also has been reported that this treatment does not alleviate scaliness in shaved EFAD rats [12]. In contrast, columbinic acid [C18:3,(n-6,9,13-trans)], which is not metabolized to prostaglandin E_2, reportedly repairs the epidermal barrier abnormality in EFAD animals [12–16]. Barrier repair occurs over both cyclo- and lipoxygenase blockade, where regulatory eicosanoids are not generated [3,17,18].

EFAD affects not only epidermal DNA synthesis but also epidermal lipid synthesis. Prior studies have shown that in EFAD animals the synthesis of both cholesterol and free fatty acids is increased, an alteration that is reversed when the barrier is artificially restored by occlusion [19]. The relationship to the permeability barrier was

Manuscript received October 29, 1991; accepted for publication March 9, 1992.

Reprint requests to: Dr. Ehrhardt Proksch, Department of Dermatology, University of Kiel, Schittenhelmstrasse 7, W-2300 Kiel 1, Germany.

These studies were supported by National Institutes of Health grant AR 19098 and the Medical Research Service, Veterans Administration. Dr. Proksch is a recipient of grants from the Deutsche Forschungsgemeinschaft Bonn-Bad Godesberg, and the Paul G. Unna Foundation, Düsseldorf, Germany.

Abbreviations:
DTT: dithiotreitol
EDTA: ethylenediamine tetraacetic acid
EFAD: essential fatty acid deficiency
HMG CoA: 3-hydroxy-3-methylglutaryl coenzyme A (reductase)
PBS: (Dulbecco's) phosphate-buffered saline
PGE_2: prostaglandin E_2
SDS: sodium-dodecyl sulfate
TEWL: transepidermal water loss

0022-202X/92/$05.00

further supported by the fact that acute barrier disruption in hairless mice also results in an increase in cholesterol and fatty acid synthesis [20], which parallels the barrier abnormality [21].

The enzyme, HMG CoA reductase catalyzes the conversion of HMG CoA to mevalonic acid and, in mammalian systems, including the epidermis, it is rate limiting for cholesterol biosynthesis [22,23]. We recently have shown that HMG CoA reductase activity increases following pertubation of the permeability barrier, an increase again prevented by latex occlusion [23,24]. These results demonstrate that in the epidermis, as in other tissues, alterations in cholesterol content are primarily modulated via changes in HMG CoA reductase activity.

The purpose of this study was to examine the role of barrier function versus hyperplasia in regulating epidermal HMG CoA reductase activity. We treated EFAD animals with latex occlusion or with selected fatty acids (linoleic acid, columbinic acid, or prostaglandin E_2) and determined barrier function, protein content, and HMG CoA reductase activity. Our results demonstrate that barrier requirements regulate HMG CoA reductase in EFAD animals. Moreover, prolonged occlusion, which suppresses HMG CoA reductase, provokes a further deterioration in barrier function in EFAD animals. Finally, we have shown that the barrier abnormality in EFAD reflects a requirement for specific fatty acids in the epidermis.

MATERIALS AND METHODS

Materials Radioisotopes, i.e., (^{14}C)HMG CoA (54.2 mCi/nmol), (^{3}H)mevalonic acid (300 Ci/nmol), (^{14}C)acetate (57.5 uCi/nmol), and (^{3}H)mevalonolactone (38.8 Ci/nmol) were purchased from New England Nuclear (Boston, MA).

Anion exchange resin (AG 1-X8, formate form, 200–400 mesh styrene-divinyl benzene matrix) was purchased from Bio-Rad Laboratories (Richmond, CA). EDTA, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (400 U/mg protein), NADP, and dithiotreitol (DTT) were purchased from Sigma Chemical Co. (St. Louis, MO). Hairless male weanling mice (HR/HR) aged 19–21 d, and weighing 6–12 g, were purchased from Jackson Laboratories (Bar Harbor, ME). The animals were divided into two groups and kept in adjacent cages on a normal light cycle room in the animal facility at the Veterans Administration Medical Center, San Francisco. Control animals were fed a diet consisting of casein, sucrose, choline, a mixture of salt, fat-soluble vitamins A, D, E, and water-soluble B vitamins to which 5% corn oil and inositol (1 mg/g) was added, whereas the EFAD group received an identical diet, except that 5% hydrogenated coconut oil was substituted for the corn oil (source: Williams MA, Tinow J, Hinzenbergs J, Thomas B, Department of Nutritional Sciences, University of California, Berkeley). TEWL was measured weekly. Mice were maintained on an EFAD diet [2] for 7–8 weeks until TEWL levels were over 10 g/m^2/h. Replenished animals were also fed the EFAD diet, supplemented with corn oil (50% linoleic acid) 4 d before the study, ad libidum.

Methods To assess directly the effect of occlusion, which instantly lowers TEWL rates to zero, groups of EFAD-treated mice were covered with a tightly fitted, water-impermeable membrane (one finger of a latex glove) for 3 d until just before the animals were killed. TEWL was measured in ether-anesthetized animals under ambient atmospheric condition, in the morning with a Meeco electrolytic water analyzer (Warrington, PA) [25]. "Ultrapure" dry nitrogen gas (99.99%) was passed through the sample cup at 100 cc/min. The sampling cup was separated from its Parafilm cover, and slid onto the site to be measured, thus minimizing exposure to atmospheric humidity. Contralateral sites were measured on each animal to ensure the reproducibility of the TEWL measurements. As in prior studies on EFAD rodents [5,22,23,26,27], TEWL rates were virtually identical over all sites on each animal, although there was considerable animal-to-animal variation.

Occlusion of animal skin with a latex wrap for 3 d results in hyperhydration of the stratum corneum, which leads to an initially high rate of water loss from the skin immediately after removal of the wrap as the excess water is lost from the stratum corneum. Therefore, the wrap is removed 5 min prior to the determination of TEWL to allow evaporation of excess water. It takes another 5 min to measure TEWL by the Meeco electronic water analyzer. During this time additional excess water is removed by a stream dry of nitrogen gas that passes through the sample cup and the water analyzer. Measurement is finished after the steady state is reached, which reflects the actual TEWL [25].

Topical Applications of Columbinic Acid and Prostaglandin E_2: In these experiments, one side of the deficient animals (6 cm^2) received once daily applications over 5 d of 30 μl columbinic acid (a gift of U.M.T. Houtsmuller, Univerlever Ltd., The Netherlands) or prostaglandin E_2 (both as methyl esters, 5 mg/100 μl) in propylene glycol : ethanol (7 : 3 v/v). Care was taken to ensure that the solutions were spread evenly and did not run. TEWL determinations were made prior to the oil applications.

Tissue Preparation for Enzyme Assay: Mice were killed by cervical dislocation, and the skin was excised and immediately placed epidermis-side downward onto a covered petri dish containing crushed ice. The undersurface of the skin pieces was scraped with a sharp scalpel blade (number 15) to remove excess subcutaneous fat. Epidermis was separated from dermis by immersion in 10 mM EDTA in Ca- and Mg-free PBS, at 37°C for 40 min. After treatment, the epidermis was peeled off the dermis in one piece by gently scraping with a scalpel blade, dried on paper towels, minced in small pieces (<1 mm^3) with scissors, and stored in small plastic tubes overnight at -70°C.

Microsomal Isolation and Enzyme Assay: Four volumes of homogenization buffer (0.3 M sucrose, 10 mM mercaptoethanol, 10 mM EDTA, sodium salt, and 50 mM sodium chloride, pH 7.4) were added to the minced tissue at 4°C. Each tissue was subjected to two separate bursts with a tissue homogenizer (Polytron PCU 2 Kinematic GmbH, Lucerne, Switzerland) for 20 seconds at 80% intensity. A 20-second pause between the bursts was employed to permit cooling of the tissue. Homogenization was continued with a Fisher Sonic Dismembranator (model 300, Artec Systems Corp., Farmingdale, NY) at 35% intensity, two times for 5 seconds with a pause of 20 seconds. The homogenate was filtered through surgical gauze soaked in the homogenization buffer, and then centrifuged in a microfuge (TM 11, Beckman Instruments, Inc., Fullerton, CA) at $800 \times g$ for 15 min. The pellet was washed with one volume of homogenization buffer and recentrifuged at $800 \times g$ for 15 min.

The pooled supernatants then were centrifuged in a microfuge at $10{,}000 \times g$ for 15 min. The $10{,}000 \times g$ supernatant was then centrifuged at $100{,}000 \times g$ for 60 min in a LB-70 M ultracentrifuge using a 50.3 TI-rotor (Beckman Instruments, Inc.). The supernatant was removed and the microsomal pellet was stored overnight at -70°C.

For the enzyme and the protein assay, the microsomal pellet was resolubilized in a solution containing 20 mM imidazol and 5 mM DTT. HMG CoA reductase activity was determined, as described previously [23]. HMG CoA reductase activity was expressed as nanomoles of mevalonate synthesized per minute per milligram of protein.

Protein Assay: The protein amount was determined with a Bio-Rad Laboratories Protein Assay dye reagent [26]. The resolubilized microsomal pellet contained both soluble and insoluble protein. The Bio-Rad Protein Assay quantitates only soluble protein ([26], Bio-Rad protein assay manual instruction). We used the same homogenization, centrifugation and resolubilization procedures in every experiment; the soluble in proportion to insoluble protein did not show significant variations. Therefore, we quantified only soluble protein in epidermal samples.

Statistical Analysis Statistical significance was determined using a two-tailed Student t test. When samples were compared from the same animal, significance was determined using a paired t test.

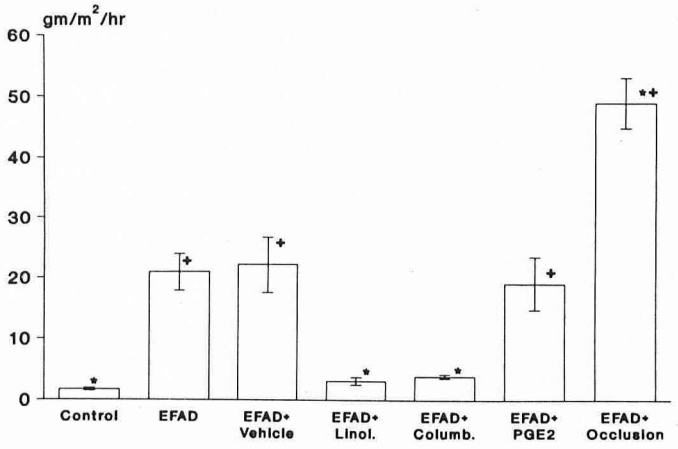

Figure 1. TEWL in EFAD. EFAD hairless mice were treated with linoleate, columbinate, prostaglandin E_2, or latex occlusion. TEWL was measured with a Meeco electronic water analyzer (n = 3–6, data are mean ± SEM *p < 0.01 versus EFAD, +p < 0.01 versus control).

RESULTS

Effects of Topical Lipids and Occlusion on Barrier Function We first measured TEWL as a marker of barrier function (Fig 1). In these studies, the essential fatty acid deficient animals demonstrated TEWL rates that were greater than 10.0 (mean 20.8 ± 3.0) g/m²/h. Use of either linoleate replenishment or topical columbinic acid for 5 d reduced the elevated TEWL rates toward normal levels (3.0 ± 0.61, n = 3, p < 0.01 and 3.84 ± 0.3 g/m²/h, n = 3, p < 0.01, respectively). In contrast, topical treatment of EFAD animals with prostaglandin E_2 (PGE_2) or the vehicle alone did not significantly affect TEWL (19.45 ± 4.35, n = 3, not significant and 22.02 ± 4.54 g/m²/h, n = 3, not significant, respectively). Providing an artificial barrier by prolonged occlusion with a latex wrap for 3 d resulted in a further increase in TEWL rates, immediately after removal of the occlusive membrane (48.73 ± 4.55 g/m²/h, n = 5, p < 0.005) respectively. These measurements indicate that the disturbed permeability barrier in EFAD mice is restored by either linoleate or columbinate replenishment, whereas PGE_2 does not repair the barrier. Moreover, prolonged latex occlusion causes a further deterioration in barrier function.

Protein Content in Treated Versus Untreated EFAD Animals Epidermal hyperplasia and increased DNA synthesis are well known features of EFAD animals. In a previous publication, we measured DNA synthesis after linoleate replenishment, topical columbinic acid and prostaglandin E_2 treatment, and latex occlusion, and observed that the restored barrier function (occlusion, columbinic acid, or linoleate) returned DNA synthesis toward normal, whereas PGE_2 had no significant effects [5]. Here, we determined protein content as a further marker of epidermal hyperplasia in a parallel cohort of animals (Fig 2). In EFAD, there was a 69% increase in epidermal protein content in comparison to normal mice (EFAD, 35.1 ± 2.4, versus normal, 20.8 ± 1.6 μg/cm², n = 5, p < 0.005). Linoleate replenishment and topical columbinic acid treatment, which repaired barrier function, reduced the quantity of protein toward normal values (24.2 ± 2.1, n = 3, p < 0.025 and 17.5 ± 1.6 μg/cm², n = 3, p < 0.005, respectively). In contrast, vehicle and prostaglandin E_2 treatment did not significantly alter protein content (33.8 ± 3.6, n = 3, not significant, and 32.2 ± 3.1 μg/cm², n = 3, not significant, respectively). Finally, prolonged occlusion with a latex wrap for 3 d markedly reduced the protein content (25.7 ± 1.7 μg/cm², n = 3, p < 0.025). These results show that linoleate replenishment, topical treatment with columbinic acid, and prolonged occlusion all markedly reduce epidermal protein content, a marker of hyperplasia in EFAD mice, whereas prostaglandin E_2 was not effective.

HMG CoA Reductase Activity in Treated Versus Untreated Animals We next determined HMG CoA reductase activity in EFAD animals treated as above. The data are expressed both as total enzyme activity (per cm²) and as enzyme-specific activity (per mg protein) (Fig 3). Total enzyme activity in EFAD mice was increased 130% in comparison to control (EFAD, 49.8 ± 2.2; control, 21.6 ± 1.6 pmol/min/cm², n = 6, p < 0.01). Latex occlusion for 3 d markedly reduced the increased enzyme activity (29.8 ± 2.8 pmol/min/cm², n = 3, p < 0.005). Linoleate replenishment and topical columbinate also reduced enzyme total activity (linoleate, 27.0 ± 4.8, n = 3, p < 0.025; columbinate, 17.0 ± 0.6 pmol/min/cm², n = 3, p < 0.001. In contrast, only a small decrease (not statistically significant) was seen after prostaglandin E_2 treatment (40.2 ± 2.4 pmol/min/cm², n = 3, not significant). The results, expressed as the specific activity of HMG CoA reductase in the entire epidermis in EFAD, were similar to the total activity values, but the differences were smaller (Fig 3*B* versus 3*A*). In EFAD epidermis, HMG CoA reductase specific activity was increased 37% in comparison to normal (EFAD, 1.42 ± 0.06; control, 1.04 ± 0.07 nmol/min/mg protein, n = 6, p < 0.025). Latex occlusion for 3 d markedly reduced enzyme-specific activity (1.16 ± 0.05 nmol/min/mg protein, n = 5, p < 0.025). Both oral linoleate replenishment and topical columbinate also reduced HMG CoA specific activity toward normal (linoleate, 1.11 ± 0.07, n = 3, p < 0.025; columbinate, 0.97 ± 0.07 nmol/min/mg protein, n = 3, p < 0.025). In contrast, prostaglandin E_2 did not significantly affect enzyme-specific activity (1.25 + 0.09 nmol/min/mg protein, n = 3, not significant). These results demonstrate that barrier restoration and reversal of hyperplasia by occlusion, linoleate, and columbinate markedly reduces the increase in HMG CoA reductase total and specific activity. In contrast, prostaglandin E_2, which did not restore barrier function or reverse hyperplasia, does not influence enzyme activity.

DISCUSSION

Role of Cholesterol and Fatty Acids in the Barrier Previous studies from our laboratory have shown that there is a relationship between barrier function and epidermal cholesterol synthesis, modulated by the rate-limiting enzyme of cholesterol synthesis, HMG CoA reductase activity (reviewed in [27]). When barrier function is acutely disturbed with either acetone or sodium-dodecyl sulfate (SDS), epidermal cholesterol synthesis and HMG CoA reductase activity both increase. Moreover, artificial restoration of the barrier by occlusion prevents the increase in both cholesterol synthesis and

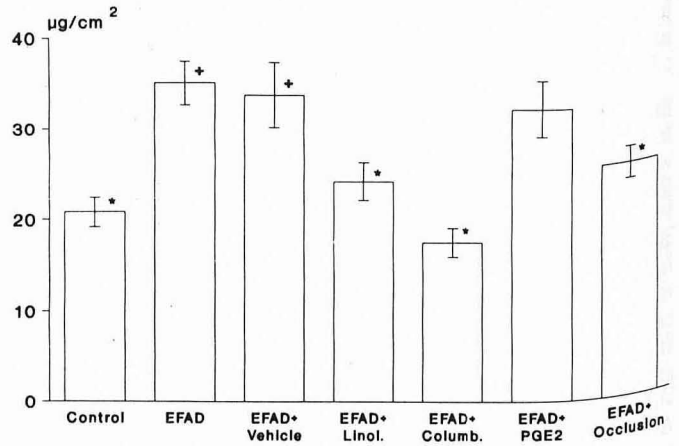

Figure 2. Protein content in EFAD. EFAD hairless mice were treated as described in Fig 1. The skin was excised and epidermis was separated from dermis by the EDTA method. After homogenization, epidermal protein content was determined as described in *Materials and Methods* (n = 3–6, data are mean ± SEM, *p < 0.025 versus EFAD, +p < 0.025 versus control).

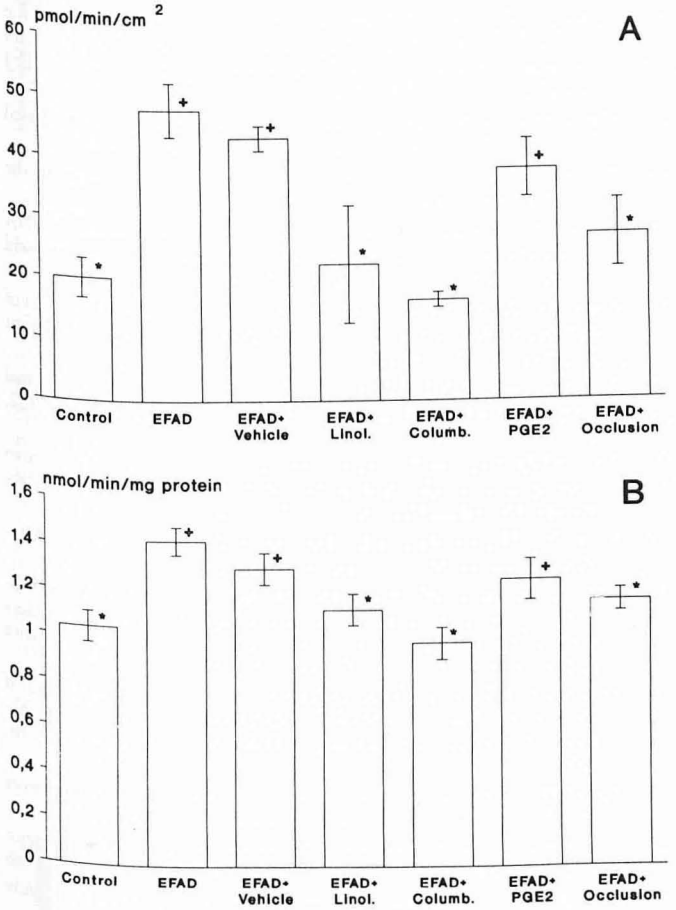

Figure 3. HMG CoA reductase total and specific activity in EFAD. EFAD hairless mice were treated as described in Fig 1. The skin was excised and epidermis was separated from dermis by the EDTA method. After homogenization HMG CoA reductase activity was determined as described in *Materials and Methods.* Total activity *(A)* was expressed per surface area (per cm^2) and specific activity *(B)* was expressed per mg protein. (n = 3–6, data are mean ± SEM, $^*p < 0.025$ versus EFAD, $^+p < 0.025$ versus control).

enzyme activity [19,23,24]. We also have shown that cholesterol synthesis is increased in EFAD, a condition that is associated with both an abnormality in barrier function [2,3,20,23,24] and epidermal hyperplasia [4,5].

Barrier dysfunction and epidermal hyperplasia are well-known, but separate abnormalities of the EFAD state. Because HMG CoA reductase activity is increased by 130% when expressed per cm^2, versus only 37% when expressed per milligram protein (these studies and [24]), about one third of the increase in enzyme total activity can be related to the abnormality in barrier function whereas two thirds can be attributed to the hyperplasia associated with EFAD. This conclusion is supported by our prior findings of an 83% increase in cholesterol synthesis and a 50% increase in DNA synthesis [5,23]. This is consistent with a requirement for cholesterol for both barrier (intercellular lipid enriched bilayers) and new cell membranes, secondary to increased cell turnover.

All treatments that restored barrier function in EFAD also lowered both the total and the specific activity of HMG CoA reductase. Linoleate replenishment and topical columbinate restored barrier function and returned enzyme activity to normal levels. In contrast, prostaglandin E_2, which did not repair barrier function, also did not lower HMG CoA reductase activity, consistant with its inability to repair the barrier [15]. After artificial barrier repair by latex occlusion, HMG CoA reductase activity was markedly reduced. This resulted in a striking deterioration in barrier function, suggesting that the increased HMG CoA reductase activity in EFAD is a compensatory response designed to repair barrier function. The link between barrier repair and lipid synthesis also has been demonstrated in prior studies. a) The increase in enzyme activity and lipid synthesis after acute barrier disruption by acetone treatment is prevented by latex occlusion. This inhibition of lipid synthesis prevents the characteristic recovery of barrier function [19,23,24,28]. b) Application of the HMG CoA reductase inhibitor lovastatin after acetone treatment delays barrier repair [29].

How essential fatty acids and selected derivates, such as columbinic acid, function in EFAD is not known. Summarizing studies from the laboratories of Houtsmuller, Needleman, and Ziboh, 18- or 20-carbon fatty acids with the linoleic acid structure (that means with cis double bonds between carbons 9 and 10 and carbons 12 and 13 (n-6,9 cis double bands) are able to normalize the skin in EFAD mice [12,13,16,30]. According to Houtsmuller and van der Beck, even-numbered fatty acids with n = 6,9 cis double bands are active, and this includes C14:2. Odd carbon fatty acids (C19 and C21) were also active if double bonds were in n-5,8 or in n-7,10 position [12]. Additional double bonds at the carboxyl end of the carbon chain also influenced activity in EFAD animals [13]. In a series of C18 and C20 fatty acids with cis or trans double bonds in position from delta-2 to delta-6, in addition to the linoleic acid configuration, all showed some ability to correct the barrier. But only gamma-linolenic acid [C18:3(n-6,9,12-cis)] and columbinic acid [C18:3(n-6,9,13-trans)] were as effective as linoleic acid [12–16]. Even the 12-trans or 13-cis compounds were considerably less active [13].

Alternatively, oxidized metabolites of linoleic acid and columbinic acid could be important for skin repair in EFAD. A (n-6) lipoxygenase can transform linoleic, gamma-linolenic, and columbinic acid into the corresponding 13-hydroxy unsaturated fatty acids [30–36]. It was shown that the lipoxygenase product of columbinic acid is capable of producing nearly as much resolution of the scaly dermatitis in EFAD rats as the fatty acid itself, whereas cyclooxygenase products were not effective [30]. If these 13-hydroxy unsaturated fatty acids have a special function in the regulation of differentiation, or if they possess structural function in acyl ceramide and/or acids, is currently unknown [30–36].

Relationship of Barrier Function to Epidermal Hyperplasia: In a previous study, we showed that DNA synthesis and epidermal hyperplasia also are linked to barrier function [5]. Acute barrier disruption by acetone treatment or tape-stripping stimulates DNA synthesis leading to epidermal hyperplasia. Artificial barrier repair by latex occlusion immediately after acetone treatment or tape-stripping prevents both the increase in DNA synthesis and epidermal hyperplasia. In addition, occlusion also normalizes the rates of DNA synthesis in EFAD animals, despite the presence of an ongoing deficiency state. These findings led us to hypothesize that the increase in DNA synthesis leading to epidermal hyperplasia in EFAD may be an attempt to repair the disturbed permeability barrier [5]. This conclusion is supported by recent measurements of changes in HMG CoA reductase activity in EFAD animals in different epidermal cell layers. Whereas enzyme total activity was increased in all nucleated layers, the increase in enzyme-specific activity was restricted to the upper epidermis (granular layers). In contrast, the increase in enzyme total activity in the lower epidermis was related to epidermal hyperplasia [24]. Here, we provide further evidence linking barrier function to hyperplasia. All factors that improved barrier function also reversed epidermal hyperplasia. Thus, latex occlusion, as well as both linoleate and columbinate replenishment, also reduced DNA synthesis, protein content, and epidermal hyperplasia, whereas PGE_2, which did not restore barrier function, also did not influence these parameters.

The link between HMG CoA reductase activity and DNA synthesis is well established. HMG CoA reductase regulates the biosynthesis of mevalonate, whose immediate isoprenoid products include farnesylated proteins, such as Ras oncogenes and lamin B, which are involved in growth control (reviewed in [37]). Moreover, the most

distal lipid product of HMG CoA reductase, cholesterol, is also required for cell replication [37]. Therefore, epidermal barrier function could regulate HMG CoA reductase activity both directly and as a consequence of the barrier-induced alterations in DNA synthesis. Columbinic acid (not metabolizable to prostaglandin E_2, while simultaneously repairing barrier function) normalized HMG CoA reductase activity and reversed epidermal hyperplasia, whereas prostaglandin E_2 did not have significant effects on barrier function, enzyme activity, or hyperplasia. This clearly shows that the normalization of skin function in EFAD mice is independent of prostaglandin E_2 generation, and suggests instead that correction of the barrier alone is sufficient to normalize epidermal lipid synthesis and hyperplasia.

In summary, these results show in EFAD animals that a) barrier function modulates HMG CoA reduction activity and cholesterol synthesis in EFAD animals; b) reduction of cholesterol, protein, and DNA synthesis by latex occlusion results in a further deterioration in barrier function, suggesting that the increases in synthesis are a protective, homeostatic response; and finally, c) that the barrier abnormality in EFAD reflects a requirement for specific fatty acids for the barrier, and is due neither to epidermal hyperplasia nor to decreased prostaglandin generation.

We wish to thank Barbara Brown for technical assistance, and both Ilse Brandt and Hildegard Kuhlmann for excellence in preparation of the manuscript.

REFERENCES

1. Prottey C: Essential fatty acids and the skin. Br J Dermatol 94:549–587, 1976
2. Elias PM, Brown BE: The mammalian cutaneous permeability barrier: defective barrier function in essential fatty acid deficiency correlates with abnormal intercellular lipid deposition. Lab Invest 39:574–583, 1987
3. Elias PM, Brown BE, Ziboh VA: The permeability barrier in essential fatty acid deficiency: evidence for a direct role for linoleic acid in barrier function. J Invest Dermatol 74:230–233, 1980
4. Lowe NJ, Stoughton RB: Essential fatty acid deficient hairless mouse: a model of chronic epidermal hyperproliferation. Br J Dermatol 96:155–162, 1977
5. Proksch E, Feingold KR, Mao-Qiang M, Elias PM: Barrier function regulates epidermal DNA synthesis. J Clin Invest 81:1668–1673, 1991
6. Wertz PW, Cho ES, Downing DT: Effect of essential fatty acid deficiency on the epidermal sphingolipids of the rat. Biochim Biophys Acta 753:350–355, 1983
7. Wertz PW, Swartzendruber DC, Abraham W, Madison KC, Downing DT: Essential fatty acids and epidermal integrity. Arch Dermatol 123:1381–1384, 1987
8. Sprecher HW: Biochemistry of essential fatty acids. Prog Lipid Res 20:13–22, 1981
9. Ziboh VA, Hasia SL: Effects of prostaglandin E_2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. J Lipid Res 13:458–467, 1972
10. Prottey C: Investigation of functions of essential fatty acids in the skin. Br J Dermatol 97:29–38, 1977
11. Lowe NJ: Essential fatty acid deficient hairless mouse: the effects of topical agents on the epidermis. Br J Dermatol 97:39–47, 1977
12. Houtsmuller UMT, van der Beck A: Effects of topical application of fatty acids. Prog Lipid Res 20:219–224, 1982
13. Houtsmuller UMT: Columbinic acid, a new type of essential fatty acid. Prog Lipid Res 20:889–896, 1982
14. Prottey C, Hartop PJ, Press M: Correction of the cutaneous manifestations of essential fatty acid deficiency in man by applications of sunflower-seed oil to the skin. J Inves Dermatol 64:228–234, 1975
15. Hartop PJ, Prottey C: Changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats. Br J Dermatol 95:255–264, 1976
16. Ziboh VA, Chapkin RS: Biological significance of polyunsaturated fatty acids in the skin. Arch Dermatol 123:1686a–1690, 1987
17. Prottey C: Investigations of functions of essential fatty acids in the skin. Br J Dermatol 97:29–38, 1977
18. Lowe NJ, De Quoy PR: Linoleic acid effects on epidermal DNA synthesis and cutaneous prostaglandin levels in essential fatty acid deficiency. J Invest Dermatol 70:200–203, 1987
19. Feingold KR, Brown BE, Lear SR, Moser AH, Elias PM: The effect of essential fatty acid deficiency on cutaneous sterol synthesis. J Invest Dermatol 87:588–591, 1986
20. Grubauer G, Feingold KR, Elias PM: The relationship of epidermal lipogenesis to cutaneous barrier function. J Lipid Res 28:746–752, 1987
21. Menon GK, Feingold KR, Moser AH, Brown BE, Elias PM: De novo sterologenesis in the skin. II. Regulation by cutaneous barrier requirements. J Lipid Res 26:418–427, 1985
22. Rodwell VW, Nordstrom JL, Mitschelen JH: Regulation of HMG-CoA reductase. Adv Lipid Res 14:1–74, 1976
23. Proksch E, Elias PM, Feingold KR: Regulation of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity in murine epidermis: modulation of enzyme content and activation state by barrier requirement. J Clin Invest 85:874–882, 1990
24. Proksch E, Elias PM, Feingold KR: Localization and regulation of epidermal 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity by barrier requirements. Biochim Biophys Acta 1083:71–79, 1991
25. Spruit D, Malten KE: The regeneration rate of the water vapour loss of heavily damaged skin. Dermatologica 132:115–123, 1966
26. Gotham SM, Frye PJ, Paterson WK: Measurement of soluble proteins using a modified Bradford assay. Anal Biochem 173:353–358, 1988
27. Feingold KR: Regulation and role of epidermal lipid synthesis. Adv Lipid Res 24:57–59, 1991
28. Grubauer G, Elias PM, Feingold KR: Transepidermal water loss: the signal for recovery of barrier structure and function. J Lipid Res 30:323–333, 1989
29. Feingold KR, Mao-Quiang M, Menon GK, Cho SS, Brown BE, Elias PM: Cholesterol synthesis is required for cutaneous barrier function in mice. J Clin Invest 86:1738–1745, 1990
30. Elliot WR, Morrison AR, Sprecher HW, Needleman P: The metabolic transformation of columbinic acid and the effect of topical application of the major metabolites on the rat skin. J Biol Chem 260:987–992, 1985
31. Bowser PA, Nugteren DH, White RJ, Houtsmuller UMT, Prottey C: Identification, isolation and characterization of epidermal lipids containing linoleic acid. Biochim Biophys Acta 834:419–428, 1985
32. Nugteren DH, Christ-Hazelhof E, van der Beck A, Houtsmuller UMT: Metabolism of linoleic acid and other essential fatty acids in the epidermis of the rat. Biochim Biophys Acta 834:429–436, 1985
33. Nugteren DH, Kivits GA: Conversion of linoleic acid and arachidonic acid by skin epidermal lipoxygenase. Biochim Biophys Acta 921:135–141, 1987
34. Elliot WJ, Huo ZY, Meyer PE, Echols BS, Forats T: Dose-response and time course of distribution of columbinic acid in rat skin (abstr). FASEBJ 2:A1064, 1988
35. Hansen HS, Jensen B, von Wettsein-Knowles P: Apparent in vitro retroconversion of dietary arachidonic to linoleic acid in essential fatty acid-deficient rats. Biochim Biophys Acta 878:284–287, 1986
36. Hansen HS, Jensen B: Essential function of linoleic acid esterified in acylglucosylceramide and acyl-ceramide in maintaining the epidermal water permeability barrier. Evidence from feedings studies with oleate, linoleate, arachonidate, columbinate, and alpha linolenate. Biochim Biophys Acta 834:357–363, 1985
37. Goldstein JL, Brown MS: Regulation of the mevalonate pathway. Nature 393:425–427, 1990